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Abrogation of the Interferon Response Promotes More Efficient Human Cytomegalovirus Replication

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The effect of abrogating the interferon (IFN) response on human cytomegalovirus (HCMV) replication was investigated using primary human cells engineered to block either the production of or the response to type I IFNs. In IFN-deficient cells, HCMV produced larger plaques and spread and replicated more rapidly than in parental cells. These cells demonstrate the vital role of IFNs in controlling HCMV replication and provide useful tools to investigate the IFN response to HCMV.

Type I interferons (IFNs) play a crucial role in the control of viral infection through inducing expression of a suite of interferon-stimulated genes (ISGs), with many of these ISGs exhibiting direct antiviral activity that serves to control viral replication (1, 2). IFNs can also act indirectly in the antiviral response by promoting the activation and proliferation of innate and adaptive immune effectors, including natural killer cells, dendritic cells, and T and B cells (3, 4). Human cytomegalovirus (HCMV) is a large double-stranded DNA (dsDNA) virus that is an important pathogen associated with severe morbidity and mortality in the immunosuppressed, especially in the allogeneic hematopoietic stem cell transplant (HSCT) setting (5). HCMV is also the leading infectious cause of birth defects in the developed world (6).

The importance of the IFN response in controlling cytomegalovirus infection is exemplified by the hypersensitivity of engineered mice with defects in the IFN response to murine cytomegalovirus (MCMV) replication and disease (7, 8). Although CMV encodes a number of gene functions that modulate the IFN response by inhibiting both the production of and response to IFNs (9–17), HCMV infection is still capable of inducing IFN-β, via an interferon regulatory factor 3 (IRF3)-dependent pathway, in human fibroblasts (HF) (9, 15, 18–25). Furthermore, treatment with exogenous type I and type II IFNs is known to restrict HCMV infection/replication *in vitro* and *in vivo* (9, 12, 26–28), confirming the sensitivity of HCMV to IFN-mediated control. Despite a number of reports investigating the IFN response to HCMV, the effect of abrogating the IFN response on HCMV infection and replication, to our knowledge, has not previously been investigated and was studied here using engineered cell lines.

Generation of IFN-deficient cell lines. To investigate the effect of abrogating the IFN response on HCMV replication, the known abilities of the nPro protein of bovine viral diarrhea virus (BVDV) to target IRF3 (blocking IFN-β production) (29) and of the V protein of parainfluenza virus type 5 (PIV-5) to target STAT1 (blocking IFN responsiveness) (30, 31) were utilized. Lentivirus vectors expressing the nPro and V genes, respectively, were generated as described previously (29). Primary HF from the ATCC (HFF-1) were transduced with the lentiviruses, and cells were selected using 1 μg/ml puromycin. To test whether nPro/HF could produce IFN-β in response to HCMV infection, parental HF and nPro/HF were infected with HCMV strain Merlin and IFN-β levels were measured at 24 h postinfection (p.i.) using a high-sensitivity enzyme-linked immunosorbent assay (ELISA) (PBL Assay Science). While IFN-β was induced by HCMV infection in parental HF, IFN-β protein was not detectable in nPro/HF samples (Fig. 1A). In addition, V/HF did not respond to IFNs even when stimulated with 1,000 pg/ml of IFN-β as determined by monitoring the relative mRNA levels of the ISGs protein kinase R (PKR) and ISG15 by quantitative reverse transcription-PCR (qRT-PCR) (Fig. 1B and C) using a published protocol (32). The sequences of the primers used are indicated: GAPDH-F, 5′-TGTTCGTCATGGGTGTGAAC-3′; GAPDH-R, 5′-GGTGCTAAGCAGTTGGTGGT-3′; PKR-F, 5′-GCTGAGCACAGGGCTAGAAG-3′; PKR-R, 5′-AACACCCTGGCATATAGTTGGA-3′; ISG15-F, 5′-GCGAACTCATCTTTGCCAGTA-3′; ISG15-R, 5′-AGCATCTTCACCGTCAGGTC-3′.

IFN-deficient cell lines promote enhanced viral spread. Titers of three distinct HCMV strains (Merlin, TB40/E, and FIX) were each determined in parallel on nPro/HF, V/HF, and the parental HF. Interestingly, inhibiting the IFN response did not alter the efficiency with which HCMV infection induced plaque formation (Table 1); however, it did result in an obvious increase in plaque size. To quantify plaque size, cells were infected with the same three HCMV strains (multiplicity of infection [MOI] of 0.0005) and cultured under an Avicel overlay for 7 days, then plaques were imaged using an XM10 camera on an Olympus IX51 microscope, and plaque size was measured using CellSens software (Olympus). All three viruses produced significantly larger

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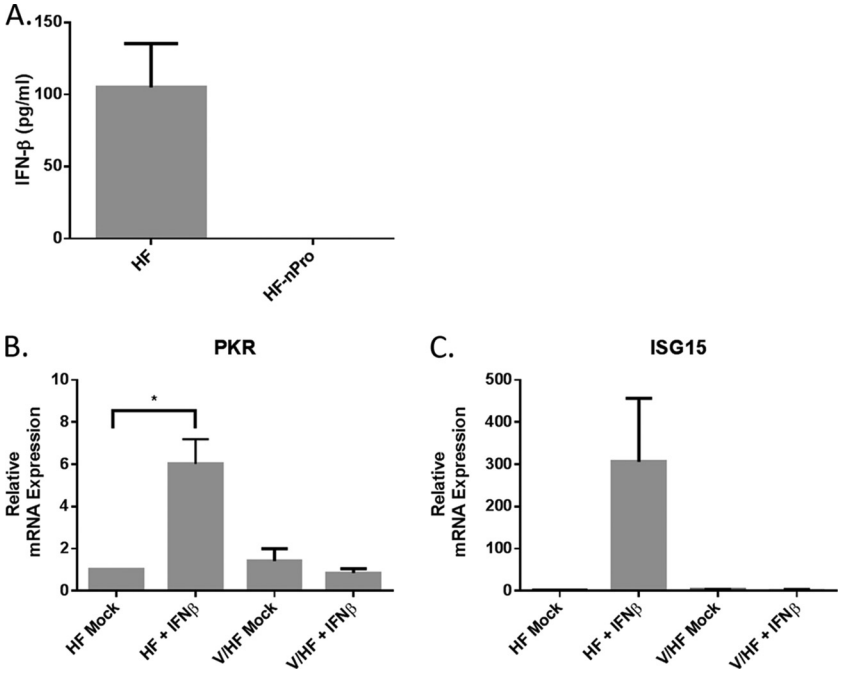

FIG 1 (A) Supernatants from HF and nPro/HF infected with the HCMV strain Merlin at an MOI of 3 were harvested at 24 h p.i. before IFN-β levels were measured by ELISA ($n = 6$ for HFF-1, $n = 3$ for nPro/HF). (B and C) HF and V/HF were mock treated or treated with 1,000 pg/ml IFN-β before relative mRNA levels of PKR (B) and ISG15 (C) were determined by qRT-PCR at 6 h posttreatment. Statistical significance compared to the parental HF cells for the V/HF cell line was tested using a two-tailed paired *t* test ($n = 3$; *, $P < 0.05$), and error bars indicate the standard errors of the means.

plaques on both nPro/HF and V/HF than on parental HF (Fig. 2A to C and E), indicating that the IFN response plays a significant role in controlling HCMV spread in fibroblasts.

A confounding issue in HCMV research has been the propensity for virus mutants, most notably in RL13 and UL128L, to be rapidly selected following *in vitro* culture (33–36), creating difficulties in culturing viruses that are genetically intact over time. HCMV variants with an intact UL128L are much more cell associated and produce smaller plaques (37). We sought to investigate whether the reduced cell-to-cell spread of UL128L intact viruses could be rescued by inhibiting the IFN response. The three cell types were transfected with a UL128L$^+$ bacterial artificial chromosome (BAC) based on the Merlin strain (pAL1160), before being cultured under an Avicel overlay. Plaques were again imaged and measured as described above (Fig. 2D). In both the IFN-deficient cell lines, there was a significant increase in plaque size over that in transfected parental HF.

HCMV replicates more efficiently on nPro/HF and V/HF. To determine if the increase in plaque size detected in nPro/HF and V/HF made a significant difference in the rate of spread and infection of HCMV, these cell types and parental HF cells were infected at an MOI of 0.01 with a BAC (pAL1158)-derived Merlin strain, expressing enhanced green fluorescent protein (EGFP) via an internal ribosome entry site (IRES) downstream of IE2 (33), before the number of GFP$^+$ cells was enumerated by flow cytometry using a FACSCanto cytometer (BD Biosciences) and FlowJo software (TreeStar Inc.) at 3-day intervals p.i. (Fig. 3A). There was a profound enhancement in the rate of viral replication and spread in both the nPro/HF and V/HF cells compared to parental HF with a significant increase in the number of GFP$^+$ cells detected at 6, 9, and 12 days p.i. ($P < 0.0001$) (Fig. 3A).

Virus production from each of the cell lines was monitored by a multistep growth curve following HCMV infection (Merlin) at an MOI of 0.01. Infectious virus released into the supernatant at 3-day intervals was measured by titration on parental HF. Consistent with the enhanced spread of virus (Fig. 3A), the production of infectious virus was also accelerated in nPro/HF and V/HF with significant differences in the amount of virus produced at both 6 and 9 days p.i. ($P < 0.05$) in the IFN-defective cells compared to parental HF (Fig. 3B). Interestingly, however, the overall peak titers of infectious virus release (day 9 for nPro/HF and V/HF; day 15 for HF) were not significantly different between each of the cell lines, indicating that abrogation of the IFN response does not affect the overall production of virus from cells but rather that the rate of replication is decreased in the presence of a functioning IFN response. We also performed similar studies using previously generated immortalized MRC5 cells engineered to express the V protein (31) infected with the Toledo/AD169 strains of HCMV. Infection of the MRC5-V cells was associated with faster spread and more rapid replication of HCMV (data not shown), indicating that enhancement of infection is not limited to a particular type of fibroblast. While it remains a possibility that the reason

TABLE 1 Titration of HCMV strains on HF, nPro/HF, and V/HF cell lines

Strain	Titer on cell line:		
	HF	nPro/HF	V/HF
Merlin	2.47×10^6	1.82×10^6	1.05×10^6
TB40/E	2.27×10^7	3.10×10^7	1.75×10^7
FIX	2.05×10^6	3.12×10^6	1.35×10^6

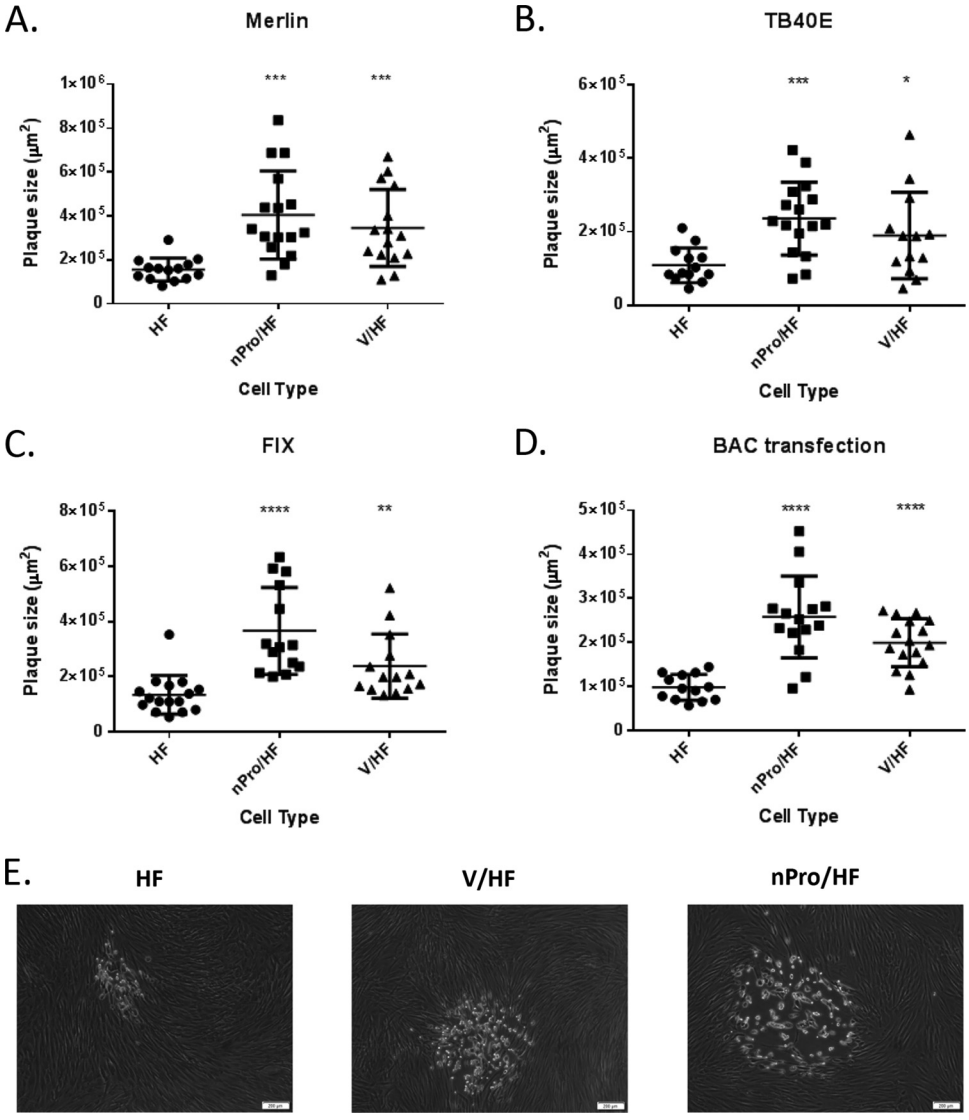

FIG 2 (A to C) The indicated cells were infected with the Merlin (A), TB40E (B), and FIX (C) HCMV strains at an MOI of 0.0005. The cells were cultured under an Avicel overlay for 7 days before plaque size was measured. (D) The indicated cells were transfected with the UL128 intact HCMV BAC (pAL1160) and cultured under an Avicel overlay for 13 days before plaque size was measured. Each data point represents an individual plaque. Statistical significance compared to the parental HF cells for both nPro/HF and V/HF cell lines was tested using a two-tailed unpaired *t* test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$). (E) Representative images of plaques formed from Merlin strain infection of HF, nPro/HF, and V/HF at 7 days p.i.

why expression of nPro can enhance CMV replication may be due to effects that it has on the cell other than blocking IFN induction, the fact that abrogating IFN signaling by expressing the V protein similarly enhances CMV replication makes this extremely unlikely.

Despite the range of proteins that HCMV encodes to inhibit the IFN response, this study demonstrates for the first time that IFN induction following HCMV infection still has a profound effect on the ability of the virus to replicate efficiently. A previous report examining the ability of V-expressing fibroblasts to enhance virus replication demonstrated a significant increase in plaque size and virus replication for other viruses, including PIV-2 and -5, adenovirus, mumps virus, measles virus, and canine distemper virus, but not for the large DNA viruses herpes simplex virus 1 (HSV-1) and vaccinia virus (31). As with HCMV, it is known that pretreatment with both type I and type II IFNs inhibits HSV-1 infection and replication (38, 39). However, in a related study examining the effect of independently derived V- and nPro-expressing fibroblasts on HSV-1 growth, there was no enhancement of viral infection/replication in IFN-deficient cell lines (39), which illustrates a significant difference between related herpesviruses on the effect of IFNs in controlling replication. Thus, the replication kinetics of other herpesviruses on these cell lines will be an important component of future studies to determine the relative importance of IFN induction in limiting their replication.

The cell lines generated will also be useful tools to examine IFN responses following HCMV and other viral infections; indeed, we have recently used these cell lines to help identify a role for HCMV-induced IFN-β in upregulating the ISG galectin-9 (32). Such cell lines can also be used to culture viruses defective in IFN

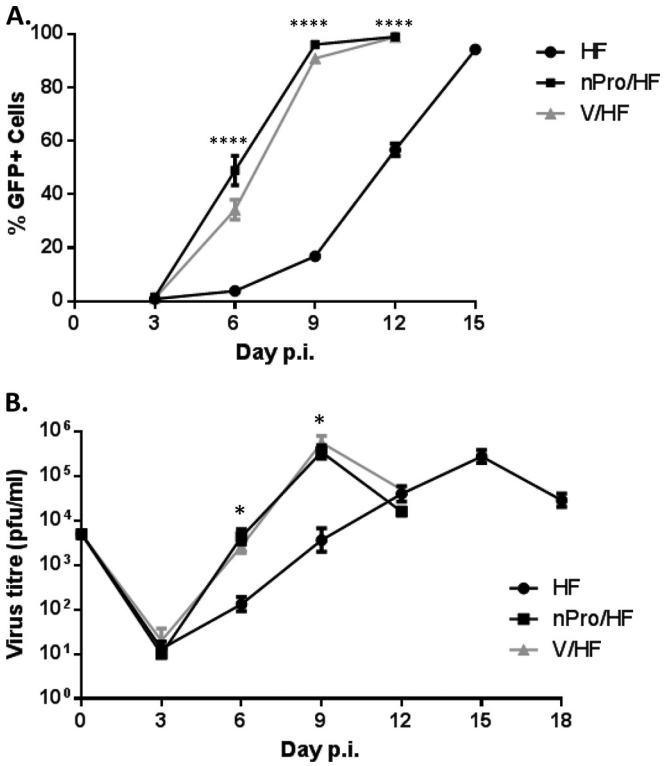

FIG 3 (A) The indicated cells were infected with HCMV-GFP (MER1158) at an MOI of 0.01, and the number of GFP^+ cells was enumerated by flow cytometry at the indicated time points. Data represent the means of 3 independent biological replicates, and error bars indicate the standard errors of the means. (B) HFF-1, nPro/HF, or V/HF were infected at an MOI of 0.01 with HCMV-GFP (MER1158) before supernatants from infected cells were harvested at 3-day intervals p.i., and titers of each of the supernatants were determined in parallel on parental HF. Data represent the means of 3 independent biological replicates, and error bars indicate the standard errors of the means. Statistical significance compared to the parental HF cells for both nPro/HF and V/HF cell lines was tested using a two-tailed paired *t* test (*, $P < 0.05$; ****, $P < 0.0001$).

regulatory genes, potentially grow UL128L intact viruses more rapidly, enhance the replication of HCMV clinical isolates directly from clinical material due to more rapid spread, and investigate the effect of individual ISGs on viral replication in the absence of a functional IFN response.

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REFERENCES

1. **Schoggins JW, MacDuff DA, Imanaka N, Gainey MD, Shrestha B, Eitson JL, Mar KB, Richardson RB, Ratushny AV, Litvak V, Dabelic R, Manicassamy B, Aitchison JD, Aderem A, Elliott RM, Garcia-Sastre A, Racaniello V, Snijder EJ, Yokoyama WM, Diamond MS, Virgin HW, Rice CM.** 2014. Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. Nature **505:**691–695. http://dx.doi.org/10.1038/nature12862.
2. **Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, Rice CM.** 2011. A diverse range of gene products are effectors of the type I interferon antiviral response. Nature **472:**481–485. http://dx.doi.org/10.1038/nature09907.
3. **Tough DF.** 2012. Modulation of T-cell function by type I interferon. Immunol Cell Biol **90:**492–497. http://dx.doi.org/10.1038/icb.2012.7.
4. **Stackaruk ML, Lee AJ, Ashkar AA.** 2013. Type I interferon regulation of natural killer cell function in primary and secondary infections. Expert Rev Vaccines **12:**875–884. http://dx.doi.org/10.1586/14760584.2013.814871.
5. **Ariza-Heredia EJ, Nesher L, Chemaly RF.** 2014. Cytomegalovirus diseases after hematopoietic stem cell transplantation: a mini-review. Cancer Lett **342:**1–8. http://dx.doi.org/10.1016/j.canlet.2013.09.004.
6. **Griffiths PD.** 2012. Burden of disease associated with human cytomegalovirus and prospects for elimination by universal immunisation. Lancet Infect Dis **12:**790–798. http://dx.doi.org/10.1016/S1473-3099(12)70197-4.
7. **Presti RM, Pollock JL, Dal Canto AJ, O'Guin AK, Virgin HW, IV.** 1998. Interferon gamma regulates acute and latent murine cytomegalovirus infection and chronic disease of the great vessels. J Exp Med **188:**577–588. http://dx.doi.org/10.1084/jem.188.3.577.
8. **Beutler B, Georgel P, Rutschmann S, Jiang Z, Croker B, Crozat K.** 2005. Genetic analysis of innate resistance to mouse cytomegalovirus (MCMV). Brief Funct Genomic Proteomic **4:**203–213. http://dx.doi.org/10.1093/bfgp/4.3.203.
9. **Taylor RT, Bresnahan WA.** 2005. Human cytomegalovirus immediate-early 2 gene expression blocks virus-induced beta interferon production. J Virol **79:**3873–3877. http://dx.doi.org/10.1128/JVI.79.6.3873-3877.2005.
10. **Tan JC, Avdic S, Cao JZ, Mocarski ES, White KL, Abendroth A, Slobedman B.** 2011. Inhibition of 2′,5′-oligoadenylate synthetase expression and function by the human cytomegalovirus ORF94 gene product. J Virol **85:**5696–5700. http://dx.doi.org/10.1128/JVI.02463-10.
11. **Li T, Chen J, Cristea IM.** 2013. Human cytomegalovirus tegument protein pUL83 inhibits IFI16-mediated DNA sensing for immune evasion. Cell Host Microbe **14:**591–599. http://dx.doi.org/10.1016/j.chom.2013.10.007.
12. **Huh YH, Kim YE, Kim ET, Park JJ, Song MJ, Zhu H, Hayward GS, Ahn JH.** 2008. Binding STAT2 by the acidic domain of human cytomegalovirus IE1 promotes viral growth and is negatively regulated by SUMO. J Virol **82:**10444–10454. http://dx.doi.org/10.1128/JVI.00833-08.
13. **Abate DA, Watanabe S, Mocarski ES.** 2004. Major human cytomegalovirus structural protein pp65 (ppUL83) prevents interferon response factor 3 activation in the interferon response. J Virol **78:**10995–11006. http://dx.doi.org/10.1128/JVI.78.20.10995-11006.2004.
14. **Spencer JV, Lockridge KM, Barry PA, Lin G, Tsang M, Penfold ME, Schall TJ.** 2002. Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. J Virol **76:**1285–1292. http://dx.doi.org/10.1128/JVI.76.3.1285-1292.2002.
15. **Hakki M, Geballe AP.** 2005. Double-stranded RNA binding by human cytomegalovirus pTRS1. J Virol **79:**7311–7318. http://dx.doi.org/10.1128/JVI.79.12.7311-7318.2005.
16. **Le VT, Trilling M, Wilborn M, Hengel H, Zimmermann A.** 2008. Human cytomegalovirus interferes with signal transducer and activator of transcription (STAT) 2 protein stability and tyrosine phosphorylation. J Gen Virol **89:**2416–2426. http://dx.doi.org/10.1099/vir.0.2008/001669-0.
17. **Zimmermann A, Trilling M, Wagner M, Wilborn M, Bubic I, Jonjic S, Koszinowski U, Hengel H.** 2005. A cytomegaloviral protein reveals a dual role for STAT2 in IFN-gamma signaling and antiviral responses. J Exp Med **201:**1543–1553. http://dx.doi.org/10.1084/jem.20041401.
18. **DeFilippis VR, Alvarado D, Sali T, Rothenburg S, Fruh K.** 2010. Human cytomegalovirus induces the interferon response via the DNA sensor ZBP1. J Virol **84:**585–598. http://dx.doi.org/10.1128/JVI.01748-09.
19. **Boehme KW, Singh J, Perry ST, Compton T.** 2004. Human cytomegalovirus elicits a coordinated cellular antiviral response via envelope glycoprotein B. J Virol **78:**1202–1211. http://dx.doi.org/10.1128/JVI.78.3.1202-1211.2004.
20. **DeFilippis VR, Robinson B, Keck TM, Hansen SG, Nelson JA, Fruh KJ.** 2006. Interferon regulatory factor 3 is necessary for induction of antiviral genes during human cytomegalovirus infection. J Virol **80:**1032–1037. http://dx.doi.org/10.1128/JVI.80.2.1032-1037.2006.
21. **Zhu H, Cong JP, Shenk T.** 1997. Use of differential display analysis to assess the effect of human cytomegalovirus infection on the accumulation of cellular RNAs: induction of interferon-responsive RNAs. Proc Natl Acad Sci U S A **94:**13985–13990. http://dx.doi.org/10.1073/pnas.94.25.13985.
22. **Browne EP, Wing B, Coleman D, Shenk T.** 2001. Altered cellular mRNA levels in human cytomegalovirus-infected fibroblasts: viral block to the accumulation of antiviral mRNAs. J Virol **75:**12319–12330. http://dx.doi.org/10.1128/JVI.75.24.12319-12330.2001.
23. **Randolph-Habecker J, Iwata M, Geballe AP, Jarrahian S, Torok-Storb**

B. 2002. Interleukin-1-mediated inhibition of cytomegalovirus replication is due to increased IFN-beta production. J Interferon Cytokine Res **22:**765–772. http://dx.doi.org/10.1089/107999002320271350.

24. **Navarro L, Mowen K, Rodems S, Weaver B, Reich N, Spector D, David M.** 1998. Cytomegalovirus activates interferon immediate-early response gene expression and an interferon regulatory factor 3-containing interferon-stimulated response element-binding complex. Mol Cell Biol **18:** 3796–3802.
25. **Preston CM, Harman AN, Nicholl MJ.** 2001. Activation of interferon response factor-3 in human cells infected with herpes simplex virus type 1 or human cytomegalovirus. J Virol **75:**8909–8916. http://dx.doi.org/10.1128/JVI.75.19.8909-8916.2001.
26. **Sainz B, Jr, LaMarca HL, Garry RF, Morris CA.** 2005. Synergistic inhibition of human cytomegalovirus replication by interferon-alpha/beta and interferon-gamma. Virol J **2:**14. http://dx.doi.org/10.1186/1743-422X-2-14.
27. **Holmes AR, Rasmussen L, Merigan TC.** 1978. Factors affecting the interferon sensitivity of human cytomegalovirus. Intervirology **9:**48–55. http://dx.doi.org/10.1159/000148920.
28. **Emodi G, O'Reilly R, Muller A, Everson LK, Binswanger U, Just M.** 1976. Effect of human exogenous leukocyte interferon in cytomegalovirus infections. J Infect Dis **133**(Suppl)**:**A199–A204. http://dx.doi.org/10.1093/infdis/133.Supplement_2.A199.
29. **Hilton L, Moganeradj K, Zhang G, Chen YH, Randall RE, McCauley JW, Goodbourn S.** 2006. The NPro product of bovine viral diarrhea virus inhibits DNA binding by interferon regulatory factor 3 and targets it for proteasomal degradation. J Virol **80:**11723–11732. http://dx.doi.org/10.1128/JVI.01145-06.
30. **Andrejeva J, Young DF, Goodbourn S, Randall RE.** 2002. Degradation of STAT1 and STAT2 by the V proteins of simian virus 5 and human parainfluenza virus type 2, respectively: consequences for virus replication in the presence of alpha/beta and gamma interferons. J Virol **76:**2159–2167. http://dx.doi.org/10.1128/jvi.76.5.2159-2167.2002.
31. **Young DF, Andrejeva L, Livingstone A, Goodbourn S, Lamb RA, Collins PL, Elliott RM, Randall RE.** 2003. Virus replication in engineered human cells that do not respond to interferons. J Virol **77:**2174–2181. http://dx.doi.org/10.1128/JVI.77.3.2174-2181.2003.
32. **McSharry BP, Forbes SK, Cao JZ, Avdic S, Machala EA, Gottlieb DJ, Abendroth A, Slobedman B.** 2014. Human cytomegalovirus upregulates expression of the lectin galectin 9 via induction of beta interferon. J Virol **88:**10990–10994. http://dx.doi.org/10.1128/JVI.01259-14.
33. **Stanton RJ, Baluchova K, Dargan DJ, Cunningham C, Sheehy O, Seirafian S, McSharry BP, Neale ML, Davies JA, Tomasec P, Davison AJ, Wilkinson GW.** 2010. Reconstruction of the complete human cytomegalovirus genome in a BAC reveals RL13 to be a potent inhibitor of replication. J Clin Invest **120:**3191–3208. http://dx.doi.org/10.1172/JCI42955.
34. **Dargan DJ, Douglas E, Cunningham C, Jamieson F, Stanton RJ, Baluchova K, McSharry BP, Tomasec P, Emery VC, Percivalle E, Sarasini A, Gerna G, Wilkinson GW, Davison AJ.** 2010. Sequential mutations associated with adaptation of human cytomegalovirus to growth in cell culture. J Gen Virol **91:**1535–1546. http://dx.doi.org/10.1099/vir.0.018994-0.
35. **Hahn G, Revello MG, Patrone M, Percivalle E, Campanini G, Sarasini A, Wagner M, Gallina A, Milanesi G, Koszinowski U, Baldanti F, Gerna G.** 2004. Human cytomegalovirus UL131–128 genes are indispensable for virus growth in endothelial cells and virus transfer to leukocytes. J Virol **78:**10023–10033. http://dx.doi.org/10.1128/JVI.78.18.10023-10033.2004.
36. **Akter P, Cunningham C, McSharry BP, Dolan A, Addison C, Dargan DJ, Hassan-Walker AF, Emery VC, Griffiths PD, Wilkinson GW, Davison AJ.** 2003. Two novel spliced genes in human cytomegalovirus. J Gen Virol **84:**1117–1122. http://dx.doi.org/10.1099/vir.0.18952-0.
37. **Murrell I, Tomasec P, Wilkie GS, Dargan DJ, Davison AJ, Stanton RJ.** 2013. Impact of sequence variation in the UL128 locus on production of human cytomegalovirus in fibroblast and epithelial cells. J Virol **87:**10489–10500. http://dx.doi.org/10.1128/JVI.01546-13.
38. **Sainz B, Jr, Halford WP.** 2002. Alpha/beta interferon and gamma interferon synergize to inhibit the replication of herpes simplex virus type 1. J Virol **76:**11541–11550. http://dx.doi.org/10.1128/JVI.76.22.11541-11550.2002.
39. **Everett RD, Young DF, Randall RE, Orr A.** 2008. STAT-1- and IRF-3-dependent pathways are not essential for repression of ICP0-null mutant herpes simplex virus type 1 in human fibroblasts. J Virol **82:**8871–8881. http://dx.doi.org/10.1128/JVI.00613-08.